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Effect of Strain of *Staphylococcus aureus* on Synergism with *Candida albicans* Resulting in Mouse Mortality and Morbidity

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Nine *Staphylococcus aureus* strains isolated from patients with toxic shock syndrome (TSS), two strains from non-disease-associated sources, and four strains from disease (not TSS)-associated sources were characterized for the intraperitoneal dose necessary to kill 50% of exposed animals (LD_{50}) and toxic shock toxin production and studied for synergistic effects on mouse mortality and morbidity when combined with a sublethal dose of *Candida albicans* and inoculated intraperitoneally. Representative toxic shock toxin-producing strains (free of other enterotoxins) exhibited the following unique set of characteristics when inoculated intraperitoneally into mice and compared with all other strains tested: (i) lowest virulence when inoculated alone into mice as determined by the LD_{50}; (ii) greatest synergistic decrease in LD_{50} (up to 70,000-fold as compared to up to 200-fold for other strains) when combined with *C. albicans* and injected intraperitoneally; and (iii) induced a characteristic, dose-independent, temporal death pattern in dually injected animals. When sublethal dual doses were used, animals receiving disease (TSS and not TSS)-associated *S. aureus* in combination with *C. albicans* developed symptoms, but some differences in symptomatologies, depending on the strain, were observed. The symptoms included conjunctivitis; gastrointestinal, neurological, and circulatory abnormalities; rash followed by desquamation; and patchy baldness. Although overlap in symptoms between animal treatment groups was observed, certain symptoms (neurological sequeae and petechial hemorrhages) were observed only in animals inoculated with a specific *S. aureus* strain combined with *C. albicans*. Animals receiving sublethal dual doses, which included non-disease-associated *S. aureus*, did not develop symptoms. When *Staphylococcus epidermidis* was combined with *C. albicans* and inoculated into mice, no synergistic effects on morbidity or mortality were observed.

It was reported previously that dual intraperitoneal (i.p.) inoculation of *Staphylococcus aureus* 2460 (isolated from a toxic shock syndrome [TSS] patient) and *Candida albicans* resulted in a strong synergistic effect on mouse mortality (4). As noted in that paper, the study was initially prompted by the observation that many epidemiological features of TSS mirror those of inveterate mycotic vulvovaginitis. For example, in both diseases outbreaks occur repeatedly and either immediately before the menstrual period (vulvovaginitis) (10, 14, 26) or during it (TSS) (8). In addition, although no study has been done, clinical literature notes that *S. aureus* and *Streptococcus* spp. are frequently found to accompany candidosis (16, 18), and in vitro *C. albicans* has been reported to enhance the growth of *S. aureus* (27).

The initial study demonstrated a synergistic relationship between one TSS-associated *S. aureus* strain and *C. albicans* on mortality in dually inoculated mice (4). This study was designed to examine: (i) whether the effect on mortality was general or differed depending on the source or ability of *S. aureus* strains to produce toxic shock toxin (TS toxin), and (ii) symptoms in animals given sublethal dual doses of *C. albicans* and various strains of *S. aureus*. Since TS toxin has been found to be associated with TSS strains (3, 23), all strains in this study were characterized for ability to produce this toxin. (This toxin was previously called pyrogenic exotoxin C [23] or staphylococcal enterotoxin F [3]; since they are believed to be the same toxin, they are now both referred to as TS toxin [M. S. Bergdoll, manuscript submitted].)

MATERIALS AND METHODS

Mice. Inbred CD-1 mice were obtained from Charles River Laboratories, Wilmington, Mass. Mice weighing between 22 and 25 g were used, with inoculated mice caged in groups of four.

TABLE 1. Characteristics[a] of *S. aureus*[b] strains

Strain	TS toxin and enterotoxins[c]	Phage type	Phage group	LD_{50} (CFU)
TSS associated				
FRI-1169	TS toxin	29/52/80 47/53/54 75/85	I, III	1.6×10^{10}
FRI-1188	TS toxin	29/52/81	I	1.3×10^{10}
2460	TS toxin, SEA	52/80	I	5.0×10^{9}
TSS1	TS toxin, SEA	29/52/80	I	8.0×10^{9}
TSS55	TS toxin	29/52/80	I	2.3×10^{9}
TSS56	—[d]	29/52	I	2.6×10^{9}
TSS62	TS toxin	81	Miscellaneous	2.1×10^{10}
TSS67	TS toxin	81	Miscellaneous	4.1×10^{9}
TSS69	SEB	96	Miscellaneous	7.4×10^{8}
Non-disease associated				
ATCC 25923	—	29/52/52A 80/81/75	I	1.0×10^{10}
M-1	ND[e]	—	Untypeable	8.4×10^{9}
Disease (not TSS) associated				
FRI-1220	TS toxin	79	I	1.3×10^{10}
C-1	—	55/71	II	7.3×10^{8}
C-2	—	42E/47/53/54 75/77/81/83A 84/85	II, III	2.0×10^{9}
C-3	—	97/96	Miscellaneous	2.0×10^{9}

[a] Methods of determining characteristics are given in the text.
[b] A strain of *S. epidermidis* was also used in this study and had an i.p. LD_{50} in mice of 9.5×10^{9} CFU.
[c] SEA, Staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B.
[d] —, Negative for characteristic.
[e] ND, Not determined.

Pathogens. *C. albicans* was from the microbiology laboratory stock culture collection at Michigan Technological University, Houghton, Mich., and it has an i.p. 50% lethal dose (LD_{50}) of 2.9×10^{8} CFU. *S. aureus* strains (2460 and strains denoted by TSS plus a number) associated with TSS were received from the Michigan Department of Public Health, which obtained them from Michigan hospitals. Other TSS-associated strains (FRI-1169 and FRI-1188) were obtained from the collection of M. S. Bergdoll (University of Wisconsin, Madison). All TSS-associated strains were isolated from patients with confirmed TSS according to the criteria of the Centers for Disease Control (6). Strains from sources associated with disease (not TSS), C-1, C-2, and C-3, were isolated in hospitals and obtained from the Michigan Department of Public Health. *S. aureus* C-1 was originally isolated from an infant with "scalded" rash syndrome, and *S. aureus* C-3 was originally isolated from a surgery-associated infection. *S. aureus* FRI-1220 was obtained from the collection of M. S. Bergdoll and was originally isolated from a food poisoning outbreak. Strains from sources not associated with disease include *S. aureus* 25923, originally obtained from the American Type Culture Collection (Rockville, Md.) but passaged many times in the laboratory, M-1, isolated in the Michigan Technological University laboratories from a mouse, and *Staphylococcus epidermidis* from the stock culture collection at Michigan Technological University. TSS-associated strains are listed in Table 1 along with phage sensitivity patterns and TS toxin and enterotoxin production. An attempt was made to use a variety of strains, as indicated by phage sensitivity pattern, virulence to mice, source, and toxin production. All strains were hemolytic on 5% sheep blood agar plates.

Bacteriophage typing. Bacteriophage typing of *S. aureus* isolates was done by the Michigan Department of Public Health under the direction of R. Martin. The phages used included: group 1, 29, 52, 52A, 79, and 80; group 2, 3A, 3C, 55, and 71; group 3, 6, 42A, 47, 53, 54, 76, 77, 83A, 84, and 85; and miscellaneous, 81, 94, 95, 96, and 187.

Pathogen injections. Organisms were introduced i.p., with each desired dose suspended in 0.2 ml of nonpyrogenic saline (Abbott Laboratories) and mixed immediately before injection. When only one agent was used, 0.2 ml of saline was substituted for the second agent. In LD_{50} studies, injected animals were observed every 2 h (except for an 8-h overnight period) for death for 5 days. Experiments to examine temporal analysis of mortality were initiated at such a time as to anticipate daytime deaths, since preliminary findings indicated that animals dually infected with the fungus and a disease-associated *S. aureus* strain died nearly synchronously. However, since animals receiving dual infections involving non-disease-associated strains died sporadically over a 3-day period, deaths were tallied every 12 h. Animals given sublethal doses and observed for symptoms were clipped with an electric hair clipper (model 27409; Racine Clipper Co., Milwaukee, Wis.) free of hair on their backs before injections and trimmed 1 week later. These animals were observed for death and symptoms for 6 weeks after inoculation.

Animal tissues. Animals were sacrificed (chloroform) at various times as indicated by the experimental protocol after injections; organs were sterilely removed and homogenized, and CFU of pathogens were determined by dilution onto selective media as described previously (5).

Identification tests included a coagulase test for *S. aureus* and the Enterotube II computer coding and identification system for gram-negative bacteria (Roche), with confirmatory tests as recommended by this system.

Streptococci were presumptively grouped by standard techniques involving hemolysis, bacitracin sensitivity, bile-esculine hydrolysis, tolerance to 6.5% NaCl, and hippurate hydrolysis.

LD_{50}. The i.p. LD_{50} was determined by the moving average method (2) for each strain of *S. aureus* by the standard procedure described previously (4). Groups of six or more animals were given doses of each *S. aureus* strain to determine at least one dose which resulted in no mortality, one dose giving complete mortality, and two doses yielding partial mortality. Where no dose tested yielded a non-mortality group, probit analysis was employed to determine the LD_{50} (12).

TS toxin. The presence of TS toxin and enterotoxins was determined under the direction of J. J. Kirkland (manuscript describing method submitted for publication) at the Procter and Gamble Co., Miami Laboratories, Cincinnati, Ohio, and M. S. Bergdoll, University of Wisconsin, Madison, by methods previously described (20).

RESULTS

Strains of *S. aureus* used, phage senitivities, production of TS toxin, enterotoxins A to E, and i.p. LD_{50} for mice are given in Table 1. Seven of the nine TSS-associated strains produced TS toxin, whereas only one of the five non-TSS-associated tested did. Four of the strains producing TS toxin and no other enterotoxin were among the most nonvirulent as judged by the i.p. LD_{50} in mice, which was greater than 10^{10} CFU. This LD_{50} is comparable to that of the *S. aureus* strains from nondisease sources and the *S. epidermidis* strain tested.

The effect on mouse mortality of combined doses of *C. albicans* and *S. aureus* is shown in Table 2. Doses could be determined for both TSS-associated and non-TSS-associated strains of *S. aureus* which caused little or no mortality alone and 100% or near 100% mortality in combination with nonlethal doses of *C. albicans*, whereas the *S. epidermidis* strain did not exhibit this effect. Sacrifice of animals injected with *C. albicans* (10^8 CFU) and *S. epidermidis* (10^9 CFU) 5 days after injection revealed bacterial infection in the abdominal organs sampled (liver, 10^5 CFU; pancreas, 10^6 CFU). *S. epidermidis* could not be recovered from these organs in mice inoculated with an identical dose of *S. epidermidis* alone. Figure 1 gives a temporal mortality analysis of six representative dual-dose experiments employing two TSS-, two nondisease-, and two disease (not TSS)-associated *S. aureus* strains. It can be seen that the fungal-bacterial combinations employing TSS-associated bacterial strains resulted in nearly synchronous animal deaths between 35 and 45 h after inoculation; non-disease-associated strains caused sporadic deaths over a 3-day period, and disease (not TSS)-associated strains resulted in 100% mortality in less than 12 h. In the experiments shown in Fig. 1, only the TSS-associated strains produced TS toxin. The temporal death pattern in animals infected with *C. albicans* and the TSS-associated strains which did not produce TS toxin (TSS56 and TSS69) resembled that of the other strains isolated from human (not TSS) disease, with an average time between the fungal-bacterial injection and death of 11 and 14 h, respectively (Table 2). The one TS toxin-producing strain not associated with TSS, FRI-1220, caused, with *C. albicans*, animal death in an average of 39 h (Table 2), which was similar to the time of deaths caused by other TS toxin-producing strains in combination with *C. albicans*. (TS toxin-producing strains, when injected in lethal amounts alone into mice, produced sporadic deaths throughout the 3-day period after injection, whereas all 7 of 10 animals dying

TABLE 2. Effect of combined i.p. doses[a] of *S. aureus* strains and *C. albicans* on mortality in mice

Strain	Dose of *C. albicans* (CFU)	Dose of *S. aureus* (CFU)	No. of dead mice/total[b]
S. aureus			
FRI-1169	1.0×10^8	5.0×10^8	6/6 (42)
FRI-1188	1.0×10^8	5.0×10^8	6/6 (38)
2460	1.0×10^8	1.4×10^9	6/6 (45)
TSS1	8.0×10^7	2.6×10^8	5/6 (33)
TSS55	1.0×10^8	8.0×10^8	6/6 (30)
TSS56	1.1×10^8	8.0×10^8	5/6 (11)
TSS62	1.0×10^8	1.0×10^9	10/12 (40)
TSS67	1.0×10^8	7.0×10^8	10/12 (37)
TSS69	1.1×10^8	2.0×10^8	5/6 (14)
ATCC 25923	1.0×10^8	3.6×10^9	5/6 (39)
M-1	1.1×10^8	8.0×10^8	3/6 (56)
FRI-1220	1.0×10^8	1.0×10^9	6/6 (39)
C-1	1.0×10^8	3.0×10^9	5/6 (13)
C-2	1.0×10^8	4.0×10^8	6/6 (12)
C-3	1.0×10^8	5.0×10^8	5/6 (8)
S. epidermidis	1.0×10^8	8.0×10^9	2/12 (51)

[a] No animals were killed by identical doses of either *C. albicans* or *S. aureus* except 1 of 12 receiving *S. aureus* TSS67 alone and 1 of 6 receiving *S. epidermidis* alone.

[b] Numbers in parentheses indicate average elapsed time in hours between inoculation and death.

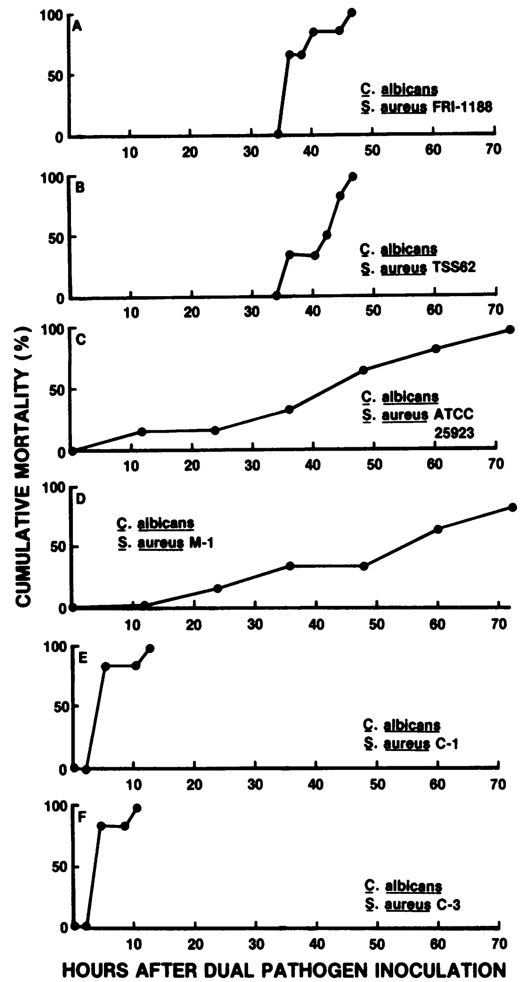

FIG. 1. Cumulative mortality in mice of representative experimental groups shown in Table 2. Mice were injected i.p. with nearly ¼ the LD_{50} of each *S. aureus* strain in combination with 10^8 CFU of *C. albicans*. The sources and doses (CFU) of the *S. aureus* strains were: A and B, TSS-associated FRI-1188 (5.0×10^8) and TSS62 (2.0×10^9); C and D, non-disease-associated ATCC 25923 (4.0×10^9) and M-1 (3.0×10^9); and E and F, disease (not TSS)-associated C-1 (1.0×10^8) and C-3 (5.0×10^8).

from twice the LD_{50} of *C. albicans* alone died within 12 h on days 2 and 3 after injection.) The results given were obtained with male animals, but no differences were found when experiments were repeated with females.

In another set of experiments, the effect of the size of the *S. aureus* dose in combination with *C. albicans* on mortality was examined. This was done by determining the LD_{50} in male mice of representative *S. aureus* strains in combination with 10^8 CFU of *C. albicans*. It can be seen in Table 3 that the LD_{50} of TS toxin-producing strains could be reduced by three to four orders of magnitude before its ability to kill half of the exposed mice when combined with *C. albicans* was lost, whereas the LD_{50} of non-TS toxin-producing strains was reduced by only one to two orders of magnitude before this effect was lost. The average elapsed time between injection and death was relatively unrelated to size of dose in the case of TS toxin-producing *S. aureus* strains, which could be reduced two orders of magnitude with no change in average number of hours between inoculation and death (data not shown). In the case of other strains, an indirect relationship between dose and time of death was observed. No mortality was observed in a group of six animals receiving 10^8 CFU of *C. albicans* alone.

To study morbidity in dually injected animals, females were dually injected with small doses of the two pathogens shown previously to cause partial mortality in the 5 days after injection. In all cases, 5.0×10^7 CFU of *C. albicans* was

TABLE 3. Effect of *C. albicans* on the LD_{50} of *S. aureus* in dually inoculated[a] mice

Strain	LD_{50} of *S. aureus* alone / LD_{50} of *S. aureus* + *C. albicans*
S. aureus	
FRI-1169[b]	$\frac{1.6 \times 10^{10}}{2.3 \times 10^5} \simeq 70{,}000$
FRI-1188[b]	$\frac{1.3 \times 10^{10}}{1.9 \times 10^6} \simeq 6{,}800$
TSS62[b]	$\frac{2.1 \times 10^{10}}{1.8 \times 10^7} \simeq 1{,}200$
TSS69	$\frac{7.4 \times 10^8}{2.5 \times 10^7} \simeq 30$
ATCC 25933	$\frac{1.0 \times 10^{10}}{8.8 \times 10^8} \simeq 11$
M-1	$\frac{8.4 \times 10^9}{1.1 \times 10^9} \simeq 8$
C-1	$\frac{7.3 \times 10^8}{4.5 \times 10^6} \simeq 164$
C-2	$\frac{2.0 \times 10^9}{2.9 \times 10^7} \simeq 69$
C-3	$\frac{2.0 \times 10^9}{6.2 \times 10^7} \simeq 32$
S. epidermidis	$\frac{9.5 \times 10^9}{9.5 \times 10^9} \simeq 1$

[a] Groups of animals were injected i.p. with nearly ⅓ the LD_{50} of *C. albicans* along with various doses of *S. aureus* so as to determine an LD_{50} (see text).
[b] Strain produces TS toxin.

TABLE 4. Effect of combined small i.p. doses of *S. aureus* strains (10^6 CFU) and *C. albicans* (5.0×10^7 CFU) on mortality and morbidity of mice[a]

Strain	No. of dead mice/total	Avg no. of days from injection to death	No. of mice exhibiting symptom/total						
			Body redness[b]	Desquamation[c]	Neurological sequelae[d]	Patchy baldness[e]	Conjunctivitis[f]	Gastrointestinal abnormalities[g]	Petechial hemorrhage[h]
S. aureus									
FRI-1169[i]	12/14	7	14/14	1/6	—[j]	6/6	8/14	+	—
FRI-1188	7/13	4	13/13	2/5	—	5/5	10/13	+	—
TSS55	4/10	8	2/8	2/8	—	4/8	9/9	+	—
TSS62	4/10	21	4/8	—	2/7	5/7	5/8	+	—
TSS69	3/10	15	6/9	2/8	—	4/8	4/9	+	—
C-1	7/10	3	6/8	1/3	—	1/3	3/6	+	—
C-3	4/12	8	6/12	4/8	—	8/8	—	+	4/12
C-2	6/10	2	7/7	3/4	—	4/4	2/7	+	—
ATCC 25923	1/10	15	—	—	—	—	—	—	—
S. epidermidis	0/10	—	—	—	—	—	—	—	—

[a] Fraction with symptoms is given on the basis of positive animals of those still alive when symptoms were first observed. Animals were observed for 6 weeks.
[b] Redness developing in less than 24 h after injection and observed on the shaved back of animals.
[c] Dandruff-like flaking of skin on shaved back observed 9 to 15 days after injection.
[d] Animals constantly held head and body in tilted position. Symptom was observed 3 weeks after injection.
[e] Hair shaved at time of injection did not grow at all or only in patches. Baldness was scored 4 weeks after clipping.
[f] Symptom was observed during the first 2 days.
[g] Symptom of diarrhea was observed during first 2 days; this was frequently followed by the appearance of constipation. Due to difficulty in scoring individual animals, + was used to indicate the presence of this symptom in the treatment group.
[h] Symptom was observed on feet, ears, and tail between 9 and 11 days, resulting in death within 48 h after appearance. Further evidence of vascular failure was indicated by accumulation of uncontaminated fluid in the abdomen.
[i] Bacterial dose in this case was 5×10^4 CFU.
[j] —, Symptom not observed.

combined with 10^6 CFU of each of the representative strains of *S. aureus* (except strain FRI-1169, for which a dose of 5.0×10^4 CFU was used) and inoculated i.p. These animals were closely observed for morbidity and mortality for 6 weeks. The findings are summarized in Table 4. Although some symptoms varied according to *S. aureus* strain, all animals for which the *S. aureus* strain was disease associated (TSS or not TSS) showed body redness in the shaved area of the back, gastrointestinal abnormalities (diarrhea followed by constipation) within 24 h of injections, patchy baldness (hair did not grow back normally, with animals still bald or showing patchy tufts of hair 1 month after last clipping), and some degree of mortality during the course of the observations. Animal groups receiving *C. albicans* and non-disease-associated *S. aureus* ATCC 25923 or *S. epidermidis* exhibited no symptoms. Certain *C. albicans-S. aureus* strain combinations caused flaking desquamation of the shaved back skin between 9 and 15 days, neurological sequelae (3 to 5 weeks after inoculation, animals exhibited a constantly tilted head and body), and petechial hemorrhages (small hemorrhages easily visible on feet, ears, tail, and shaved backed area appearing 6 to 10 days after injection). Animals developing petechial hemorrhages died within 3 days after this symptom was observed.

Control animals receiving 10^6 CFU i.p. of *S. aureus* alone showed no symptoms. Nearly 50% of the animals receiving 5.0×10^7 CFU of *C. albicans* i.p. showed body redness and gastrointestinal abnormalities, although to a lesser extent than animals receiving dual pathogen injections. No other symptoms were observed in control animals receiving either pathogen alone at these doses.

Two months after injections, seven representative animals were sacrificed from dually injected groups, and organs and tissues were examined to determine titers of *S. aureus*, *C. albicans*, and other bacteria. Infections due to the injected pathogens were found in abscesses associated with the liver and pancreas in all animals examined. Abscesses from two of the seven also contained additional organisms. Bacteria found along with *S. aureus* and *C. albicans* were *Streptococcus* group D, *Streptococcus* he-

TABLE 5. Effect of time of i.p. injection of various *S. aureus* strains on mortality of *C. albicans*-infected mice[a]

Experimental group	Dose (CFU)	No. of dead mice/total after the following times (h) from injection of *C. albicans* until injection of *S. aureus*				
		0	2	12	24	48
C. albicans	1.0×10^8	6/6	3/6	2/6	2/6	0/6
S. aureus 2460	1.0×10^9					
C. albicans	1.0×10^8	6/6	6/6	1/6	2/6	0/6
S. aureus TSS62	1.5×10^9					
C. albicans	1.0×10^8	6/6	6/6	5/6	6/6	0/6
S. aureus ATCC 25923	4.0×10^9					
C. albicans	1.0×10^8	6/6	6/6	2/6	2/6	0/6
S. aureus C-1	1.5×10^8					
C. albicans	1.0×10^8	6/6	6/6	3/6	2/6	0/6
S. aureus C-3	5.0×10^8					

[a] Experiments were followed 5 days after last injection.

molytic non-group D, and *Escherichia coli*. (Animals examined as long as 3 months after injection have been found to still harbor the injected pathogens.) The phage sensitivity type of the recovered *S. aureus* matched that of the bacteria originally injected.

To examine the possibility of sex differences, the effects of a sublethal dose of *C. albicans* (10^8 CFU) and *S. aureus* C-3 (10^6 CFU) on a group of 10 female mice was compared with a group of 10 male mice with identical treatment. Both male and female animal groups showed similar mortality, petechial hemorrhages, and other symptoms, except that whereas 50% of the females showed patchy baldness, no males exhibited this symptom. This sex-associated difference was reproducible in five subsequent trials with different *S. aureus* strains.

Experiments were also conducted to determine how long after *C. albicans* infection various *S. aureus* strains could be added before the synergistic effect on mouse mortality was destroyed. Table 5 shows that for all *S. aureus* strains, the effect decreased with time and was lost completely when *S. aureus* injection followed *C. albicans* by 48 h.

DISCUSSION

Schlievert et al. (23) and Bergdoll et al. (3) have found a high degree of association between the presence of TS toxin and TSS involvement of *S. aureus* strains. However, the role of this toxin in the disease process remains obscure. In rabbits, using injection of the bacteria into subcutaneous chambers to assess the virulence, TSS strains proved more virulent than non-TSS strains (24). Meanwhile, Barbour (1), using culture filtrates, found TSS strains less toxic than non-TSS strains to both chicken embryos and rabbits when injected intravenously.

In the experimental mouse system reported here, TS toxin-producing TSS strains (producing no other enterotoxins), inoculated i.p., were found to be among the least virulent of the strains tested. TS toxin-producing TSS strains of a greater virulence, equivalent to disease (not TSS) strains, all produced additional enterotoxins or, in the case of *S. aureus* TSS55 and TSS67, probably elaborated more hemolysins, as indicated by appearance on sheep blood agar. Therefore, it is concluded that strains which produce TS toxin in the absence of other toxins are of low virulence to mice due either to lack of toxin production in the experimental system or to the need for some additional underlying condition or activating factor. Since in the presence of *C. albicans* these strains are among the most lethal, it seems reasonable to conclude that TS toxin is probably toxic to mice, at least under certain conditions, although a role of hemolysins in morbidity and mortality cannot be ruled out.

Kapral (15) reported that TSS-associated strains of *S. aureus* have a hemolysin pattern similar to that of other strains, whereas Chow et al. (7) found that the vaginal TSS isolates were more likely to produce δ-hemolysin alone and also differed as a group by producing smaller amounts of all hemolysins. The exceptionally high LD_{50}s of TS toxin-producing strains suggest a lack of hemolysin production in mice infected with these strains alone, but one could argue that the presence of *C. albicans* could influence hemolysin levels. Studies are presently in progress to characterize the strains used in this study for hemolysin production and to test for synergistic effects between *C. albicans* and purified TS toxin as well as other enterotoxins and hemolysins.

The finding that all *S. aureus* strains acted synergistically with *C. albicans* to cause animal mortality was not unexpected in that we have found previously that candidal stimulation of bacterial infection is a general effect (5). What is new and of interest in this study is that TS toxin-producing strains interacted with *C. albicans* to a far greater extent in this respect, with LD_{50}s dropping in the presence of nonlethal doses of this fungus by three to four orders of magnitude, whereas LD_{50}s of all other strains fell by only two orders of magnitude or less. It is interesting to note that previous reports have described a synergistic effect between *C. albicans* and gram-negative organisms on mouse mortality, but this effect could not be reproduced with the gram-positive bacteria *S. aureus*, *Streptococcus* spp., and *Bacillus subtilis* (28). It was therefore proposed that this bacterial-fungal synergism required gram-negative organisms (for a review, see reference 9). This apparent contradiction between our work and past findings could be explained by the difference in amount of synergism with *C. albicans* exhibited by various strains and species of *Staphylococcus*. Our findings further suggest that candidal stimulation of infecting bacterial is a general effect, but the degree of resulting mortality will depend on the array of toxins produced by the multiplying bacteria.

The disproportionally large stimulation of virulence by *C. albicans* of TS toxin-producing *S. aureus* strains and the unique dose-independent temporal death pattern in these dual infections suggest that *C. albicans* interacts with these strains in some way in addition to simple amplification of infecting numbers. Indeed, dual infections involving non-TSS-producing *S. aureus* of a low virulence equivalent to that of the TS toxin-producing strains resulted in no mortality increase when the bacterial dose was decreased to less than 1/10 its LD_{50} when inoculated alone.

A quantitative analysis of TS toxin production has not been done for the strains used in this study. It is noteworthy, however, that *S. aureus* FRI-1169, whose virulence was most stimulated by *C. albicans*, has been found to produce 5 to 10 times more TS toxin than the other TS toxin-producing strains tested (M. S. Bergdoll, personal communication).

Endotoxins have been proposed to play a role in TSS (21). Pyrogenic exotoxin C (believed to be the same toxin as staphylococcal enterotoxin F or TS toxin [22]) produced by TSS-associated *S. aureus* has been reported to amplify endotoxicity (17). However, a source of the endotoxins in TSS in unknown. In this experimental system, one may speculate that *C. albicans* could possibly induce an inhibition of phagocytosis which would reduce the effectiveness of the elimination of the normal levels of endotoxins which is then amplified by the *S. aureus*-associated TS toxin. In addition, the ability of *C. albicans* to increase the host animals' production of histamine (19) and the ability of this substance to facilitate the passing of endotoxins from the gut into the bloodstream (11) may exacerbate the situation. Alternatively, *C. albicans* itself has been reported to produce substances with endotoxin-like properties (9, 13).

It must be emphasized that the study reported here was not designed as a model for human disease, but rather to study further the fungal-bacterial synergistic effect previously reported, and the system employed here is believed to optimize this effect. However, a number of similarities between the types of symptoms observed (conjunctivitis; gastrointestinal, neurological, and circulation abnormalities; rash followed by desquamation; and patchy baldness) in these dually infected animals and in human TSS patients (25) are noted. In addition, the impressive stimulation by *C. albicans* of virulence in the otherwise nonvirulent TS toxin-producing strains of *S. aureus* strengthens the possibility of a relationship between these organisms in disease. Thus, the combination of *S. aureus* and *C. albicans* may provide a model pathogen combination which could represent one condition leading to a unique TSS-like disease.

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LITERATURE CITED

1. **Barbour, A. G.** 1981. Vaginal isolates of *Staphylococcus aureus* associated with toxic shock syndrome. Infect. Immun. **33**:442–449.
2. **Bennet, B. M.** 1952. Estimation of LD_{50} by moving averages. J. Hyg. **50**:157–164.
3. **Bergdoll, M. S., B. A. Crass, R. F. Reiser, R. N Robbins, and J. P. Davis.** 1981. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus* isolates. Lancet **i**:1017–1021.
4. **Carlson, E.** 1982. Synergistic effect of *Candida albicans* and *Staphylococcus aureus* on mouse mortality. Infect. Immun. **38**:921–924.
5. **Carlson, E.** 1983. Enhancement by *Candida albicans* of *Staphylococcus aureus*, *Serratia marcescens*, and *Streptococcus faecalis* in the establishment of infection in the mouse. Infect. Immun. **39**:193–197.
6. **Centers for Disease Control.** 1980. Follow-up on toxic shock syndrome. Morbid. Mortal. Weekly Rep. **29**:441–445.
7. **Chow, A. W., M. J. Gribble, and K. H. Bartlett.** 1983. Characterization of the hemolytic activity of *Staphylococ-*

cus aureus strains associated with toxic shock syndrome. J. Clin. Microbiol. **17**:524–528.

8. **Davis, P., P. J. Chesney, P. J. Wand, M. LaVenture, and the Investigation and Laboratory Team.** 1980. Toxic shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. N. Engl. J. Med. **303**:1429–1435.
9. **Dobias, B.** 1964. Specific and non-specific immunity in *Candida* infections: experimental studies of the role of *Candida* cell constituents and review of the literature. Acta Med. Scand. **176**(Suppl. 421):1–79.
10. **Drake, T. E., and H. I. Maibach.** 1973. *Candida* and candidiasis. Postgrad. Med. **53**:83–87.
11. **Fine, J., and P. Cuevas.** 1973. Production of fatal endotoxic shock by vasoactive substances. Gastroenterology **69**:285–291.
12. **Finney, D. J.** 1971. Probit analysis. Cambridge University Press, New York.
13. **Hasenclever, H. F., and W. O. Mitchell.** 1963. Endotoxin-induced tolerance to toxic manifestations of *Candida albicans*. J. Bacteriol. **85**:1088–1093.
14. **Hurley, R.** 1975. Inveterate vaginal thrush. Practitioner **215**:753–756.
15. **Kapral, F.** 1982. Epidermal toxin production by *Staphylococcus aureus* strains from patients with toxic shock syndrome. Ann. Intern. Med. **96**:972–974.
16. **Kenney, E. L.** 1951. *Candida* asthma. Ann. Intern. Med. **34**:223–226.
17. **Kim, Y. B., and D. W. Watson.** 1970. A purified group A streptococcal pyrogenic exotoxin. Physicochemical and biological properties including the enhancement of susceptibility to endotoxin lethal shock. J. Exp. Med. **131**:611–628.
18. **Neufeld, O., and W. L. Wallbank.** 1952. Case of *Candida* asthma and its management. Mich. Med. **51**:1419–1420.
19. **Nosal, R.** 1974. Histamine release from isolated rat mast cells due to glycoprotein from *Candida albicans* in vitro. J. Hyg. Epidemiol. Microbiol. Immunol. **18**:337–338.
20. **Robbins, N., S. Gold, and M. Bergdoll.** 1974. Detection of enterogenicity in *Staphylococcus aureus*. Appl. Microbiol. **28**:946–950.
21. **Schlievert, P. M.** 1982. Enhancement of host susceptibility of lethal endotoxin shock by staphylococcal pyrogenic exotoxin C. Infect. Immun. **36**:275–279.
22. **Schlievert, P. M., and J. A. Kelly.** 1982. Staphylococcal pyrogenic exotoxin type C: further characterization. Ann. Intern. Med. **96**:982–986.
23. **Schlievert, P. M., K. N. Shands, B. B. Dan, G. P. Schmid, and R. D. Nishimura.** 1981. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. J. Infect. Dis. **143**:509–516.
24. **Scott, D. F., J. M. Kling, J. J. Kirkland, and G. K. Best.** 1983. Characterization of *Staphylococcus aureus* isolates from patients with toxic shock syndrome, using polyethylene infection chambers in rabbits. Infect. Immun. **39**:383–387.
25. **Tofte, R. W., and D. N. Williams.** 1982. Clinical and laboratory manifestation of toxic shock syndrome. Ann. Intern. Med. **96**:843–847.
26. **Truss, C. O.** 1978. Tissue injury induced by *Candida albicans*, mental and neurologic manifestations. J. Orthomol. Psychiatry **7**:17–37.
27. **Virtanen, I.** 1951. Observations on the symbiosis of some fungi and bacteria. Ann. Med. **29**:352–358.
28. **Yamabayaski, H.** 1958. A zymosanlike substance extracted from *Candida albicans*. Med. J. Osaka Univ. **9**:11–21.